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Early root growth and architecture of fast- and slow-growing Norway spruce (*Picea abies*) families differ – potential for functional adaptation

Running title: Root growth differs in spruce

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Keywords: seedlings, phenotypes, fine roots, WinRHIZO™, mixed models

Abstract

The relationship between the growth rate of aboveground parts of trees and fine root development is largely unknown. We investigated the early root development of fast- and slow-growing Norway spruce (*Picea abies* L.) H. Karst. families at a developmental stage when the difference in size is not yet observed. Seedling root architecture data, describing root branching, were collected with the WinRHIZO™ image analysis system, and mixed models were used to determine possible differences between the two growth phenotypes. A new approach was used to investigate the spatial extent of root properties along the whole sample root from the base of one-year-old seedlings to the most distal part of a root. The root architecture of seedlings representing fast-growing phenotypes showed ca. 30% higher numbers of root branches and tips, which resulted in larger root extensions and potentially a better ability to acquire nutrients. Seedlings of fast-growing phenotypes oriented and allocated root tips and biomass further away from the base of the seedling than those growing slowly, a possible advantage in nutrient limited and heterogeneous boreal forest soils. We conclude that a higher long-term growth rate of the aboveground parts in Norway spruce may relate to greater allocation of resources to explorative roots that confers a competitive edge during early growth phases in forest ecosystems.

Keywords: seedlings, phenotypes, fine roots, WinRHIZO™, mixed models

Introduction

The relationship between tree phenotype, root proliferation and the spatial distribution of fine roots is largely unknown. For example, in Norway spruce (*Picea abies* L.) H. Karst., some genotypes grow faster and repeatedly outcompete others in long-term field experiments, but whether this relates to root development has seldom been investigated (Korkama et al. 2006, Velmala et al. 2014a). Resource allocation to roots may be a crucial part of a tree's strategy in competition with conspecifics and other species within a forest.

Variation in early root structure, such as larger root systems, implies great differences in the potential of growing roots to acquire nutrients and water in heterogeneous forest soils that may lead to better growth in later stages of long-lived trees. Small roots, also called fine roots, are traditionally defined as roots less than 2.0 mm in diameter, but in more recent investigations only roots less than 1.0 or 0.5 mm in diameter are considered fine roots (Pregitzer 2002, Pregitzer et al. 2002, Bagniewska-Zadworna et al. 2012, Beidler et al. 2015, McCormack et al. 2015). Fine root tissue is nutritionally expensive to produce and maintain due to its high concentration of nitrogen, metabolic activity and respiration rate (Pregitzer et al. 1997, Pregitzer 2002) as well as its turnover (Leppälammi-Kujansuu et al. 2014). In young tree seedlings, two functionally different fine root types can be found, i.e., pioneer roots and fibrous roots (Zadworny and Eissenstat 2011, Bagniewska-Zadworna et al. 2012; Zadworny et al. 2017). In developing root systems, the function of pioneer roots is soil exploration and assurance of long-distance, rapid transport of water and nutrients as well as provision of a framework for the whole root system. Pioneer roots are long and thick, not colonized by mycorrhizal fungi, they grow and extend rapidly in the soil both horizontally and vertically, and branch intensively since they typically quickly produce fibrous roots responsible for absorption of water and nutrients (Zadworny and Eissenstat 2011; Bagniewska-Zadworna et al. 2012). The production of short-roots, also called root tips, or exterior links by Fitter and

Stickland (1991), and associations with mycorrhiza increase the surface-area of roots and thus the uptake of nutrients and water for a given amount of carbon (Ostonen et al. 2007, 2013). Root tips have a specific, dynamic role in root systems especially in absorbing water and acquiring nutrients, with high biomass and turnover (Wang et al. 2006).

Norway spruce adapts its root growth in response to environmental conditions (Kalliokoski et al. 2008). Stand characteristics have significant impacts on the architecture of coarse roots and affect the amount of root tips in the organic layer (Helmisaari et al. 2009, Kalliokoski et al. 2008, 2010). Furthermore, carbon allocation and the longevity of structural compounds differ with thickness and the classification of roots, implying huge functional and physiological heterogeneity within the outer rhizosphere (Keel et al. 2012).

Boukcim and Plassard (2003) showed that the number and total length of root tips were higher in a fast-growing spruce family compared to a slow-growing family. Accordingly, we have previously found some differences between seedling roots of six Norway spruce families showing contrasting growth performance in long-term field trials (Velmala et al. 2014a, b). The density of outermost ectomycorrhizal root tips (number of root tips per root length) was lower in fast-growing than slow-growing families when seedlings were still of the same size. Beyond these associations between root tips and phenotypic characteristics, a genetic component driving root tip formation and root growth in Norway spruce was found by comparing 55 clonal genotypes. The density of root tips among spruce clones varied almost twofold, indicating huge adaptive potential, with root tip density being moderately heritable ($H^2 = 0.41$) (Velmala et al. 2013).

However, evidence on the relationship between root structuring and shoot growth rate is still incomplete as spruces were not included in the study where root traits of fast- and slow-growing plants were compared (Comas and Eissenstat 2004). Bouckim and Plassard (2003) compared only two Norway spruce families, while in other spruce root

studies comparing fast- and slow-growing phenotypes (Korkama et al. 2006, 2007, Velmala et al. 2013, 2014a, b), no architectural analyses of root systems were performed. Especially, studies including complete root segments extending from the base of a seedling to the most distal part of a root are missing.

We compared the early root formation of six Norway spruce families showing contrasting growth performance in long-term field trials before any differences in aboveground growth were found. We were interested in determining whether there are differences in root biomass, spatial extension of root systems and ability to form root branches. Detailed architectural data on whole sample roots originating from the base of a seeding were used in these analyses. The data included information on all root pieces originating from each branching point. We hypothesized that seedlings of fast-growing families would display lower root tip density (i.e., number of root tips per root length, see Velmala et al. 2014a, b). Furthermore, we hypothesized that in seedlings of fast-growing families, the length of sample roots is higher and includes more side branches (see Fig. 1) further away from the base of a seedling than in seedlings of slow-growing families. To the best of our knowledge, no previous intraspecific studies have concentrated on spatial differences in tree roots, i.e., differences in root branching intensity along the length of sample roots.

Material and Methods

Seedlings

The study was performed using six Norway spruce seed orchard families that originated from southern Finland and were selected for breeding purposes (Table 1). Three families were classified as fast-growing (good and excellent growth; family codes 298, 612, 1162) and three as slow-growing families (stunted; family codes 394, 427, 1183) based on stem height

measured in several long-term field trials. These contrasting groups of spruce families are hereafter referred to as the fast- and slow-growing phenotypes.

Seeds were germinated under optimal growth conditions in nursery containers with unfertilised light *Sphagnum* peat (PP03, Kekkilä, Vantaa, Finland) in a glasshouse at the end of April 2011. Seedlings were transplanted after six weeks into Plantek-81F containers (vol. 85 cm^3, BCC) filled with sieved forest humus, which was excavated from the uppermost layer of fine sandy till of a Norway spruce stand located in southern Finland. The humus acted as a natural source of forest microbiota, e.g. ectomycorrhizal fungi. In August, sieved forest humus (30 ml) was added again to the base of each seedling. Seedlings were grown outdoors in an open forest nursery field (62.625 N, 27.122 E, Supplementary Table 1) following common seedling production practises in Finland, i.e., irrigated regularly and fertilized once a week according to the Kekkilä Forest-Superex fertilisation program (NPK 22-5-16) - each seedling was provided with approximately 10 mg N during the growing season. The seedlings overwintered outdoors under natural snow cover. In the following summer, seedlings were grown in the same open nursery field and fertilized once a week according to the Kekkilä Forest-Superex fertilisation program with a slightly higher N load: the conductivity of peat was 2 mS cm^{-1}. In early August 2012, at 16 months of age and after two growing seasons, nine replicate seedlings from each family were sampled (Fig. 1a).

Seedlings were removed from containers, the shoot was detached from roots and shoot height was measured from the base to the top of a seedling (mm) in order to examine if the phenotypes differed in height. Roots were washed gently with tap water and stored in water at +4 °C.

Description of root data

In most cases, there were altogether ca. five root branches per seedling. Three uppermost sample roots per seedling were selected for the architectural analysis by sampling down from the root collar (Fig. 1a). Thus, altogether 162 detached roots were investigated in detail. The whole sample root from the base of a seedling up to the most distant part of a root was included in the architectural analysis. Fresh sample roots were kept in water on large Petri-dishes when scanned with an Epson perfection V700 Photo scanner (SeikoEpson Corporation), and analysed using the WinRHIZO™ image analysis system professional software (2013) in order to obtain detailed link-based data for statistical analyses (see below). Here, a link is defined as a piece of root between two branches or a piece of root forming a root tip (Fig. 1b).

Soil humus ensured that the roots were highly ectomycorrhizal (>81%, Velmala et al. 2014b). On average, seedlings were colonized with ca. five ectomycorrhizal species and the fast- and slow-growing families did not differ in terms of their ectomycorrhizal communities. The most abundant ectomycorrhizal colonizers belonged to the genera *Amphinema*, *Piloderma* and *Thelephora* which comprise more than two thirds of the occurrences. The ectomycorrhizal fungal communities of the families are described in detail in Velmala et al. (2014b).

After scanning, roots and shoots were dried at 60 °C for 24 h. Total biomasses of roots and shoots (g) were determined for three seedlings per family. Dry biomass (g) was measured separately for detached sample roots.

In our data analyses, we used the developmental order-based classification of WinRHIZO^{TM} (2013) that counts root orders from the main branch of a sample root outwards instead of the functional stream-order classification beginning from the outermost roots inwards. This approach was chosen as our root systems were relatively small and simple (mean diameter < 1 mm, Table 2, McCormack et al. 2015), and because the developmental

order-based classification provides more information on root architecture (i.e., on architectural differences between the fast- and slow-growing phenotypes, such as the length of the main branch of a sample root and the number of side branches along it, see Fig. 1b) which was the main focus of the present study. In the developmental order-based classification, the main branch of a sample root originating from the base of a seedling was considered a zero order root, and at the branching point the thicker branch continued the same zero order while the thinner one belonged to the next order. Thus, a root branch originating from the zero order root was considered a first order root branch, and a root branch originating from the first order root was considered a second order root branch, etc. (Fig. 1b). The developmental order-based classification is especially useful for roots with a clear main root axis or branch, and in which clear branches of higher orders can be observed, such as in young spruce seedlings in our study (Fig. 2). Unlike the developmental order-based classification of roots used in the present study, the stream-order classification is more suitable for describing the function of roots based on the division between absorbing and transporting roots so that the first three root orders representing absorbing roots are considered anatomically and physiologically different from higher root orders (Pregitzer et al. 2002, McCormack et al. 2015, Liu et al. 2016, Norby and Iversen 2017). It seems that the functional stream-order classification has widely been used in studies where roots have been cut to small fragments (e.g., Chen et al. 2016; Ostonen et al. 2017; Zadworny et al. 2017), whereas in the present study, a whole sample root was investigated in detail. Yet, root tips, also known as exterior links, and interior links (other links than root tips) were investigated separately (Fitter and Stickland 1991) to ensure comparability to the functional classification.

In the WinRHIZO^{TM} (2013) data, the number, length (mm) and surface area (mm^2) of each link of each order were available. WinRHIZO^{TM} also calculated root volume per sample root (mm^3). Furthermore, angle, in degrees, between each pair of two links

originating from the same branching point was provided. Utilizing this information, we calculated the total number, length and surface area of links per sample root, and separately for zero to the highest order links of root tips and interior links. In addition, root tip density, i.e., the number of root tips divided by the total length of links, and the mean angle for root tips and interior links were calculated per sample root. A side branch was defined as one branch of a root that originates from the zero order root link and contains all branching links from that point (see Fig. 1b). Side branches include more than one link, and are thus separated from exterior links (i.e., root tips). Utilizing the LIGNUM software (Sievänen et al. 2010), we determined the occurrence of side branches (Fig. 1b), the number of all links and root tips and the length of all links within each branch (both side branches and root tips were considered as branches) along a zero order root. The LIGNUM software was also used to visualize the roots.

Utilizing link-based data, we calculated specific root length (SRL, ratio of root length to root dry mass, mm g^{-1}), specific root area (SRA, ratio of surface area to root dry mass, m^2 kg^{-1}) and root tissue density (RTD, ratio of root dry mass to root volume, kg m^{-3}) for all seedlings.

Statistical analyzes

Differences in shoot height (height of a seedling from the base to the top, mm), and total biomass of the three sample roots (g), specific root length (SRL, mm g^{-1}), specific root area (SRA, m^2 kg^{-1}) and root tissue density (RTD, kg m^{-3}) between the fast- and slow-growing phenotypes were analyzed using linear mixed models (LMMs) in R with the *lme* function in library *nlme* (n = 54, six spruce families with nine seedlings in each, see Ostonen et al. 2013, Maherali 2014, R Core Team 2015, Pinheiro et al. 2016, Weemstra et al. 2016). In the

LMMs, response variables were either the height, SRL, SRA or RTD of a seedling or the total biomass of three sample roots per seedling. As an explanatory variable, we used the long-term growth rate of a family (a factor with two levels: fast or slow), and as random factor we used the family (seedlings belonging to the same origin were given the same family code). Similarly, differences in the total biomass of roots and shoots, and root:shoot ratio, between seedlings of fast- and slow-growing phenotypes were investigated as above (n = 18, six spruce families with three seedlings in each).

Detailed link data for each sample root of a seedling (n = 162, i.e., 54 seedlings with three sample roots in each), produced by WinRHIZO™ (2013), were analyzed using linear (LMMs) or generalized linear mixed models (GLMMs) in R. These models were estimated separately for root volume, root tip density, and number, angle and surface area of links, measured separately for each sample root of an individual seedling. These response variables were mainly investigated for the whole sample root, and for zero, first, second and the combined third to fifth order links separately (Fig. 1). Count response models were estimated assuming a Poisson distribution with log link function using the *glmer* function in the *lme4* library (Bates et al. 2015). For other responses, a normal distribution was assumed, and the *lme* function in library *nlme* was used to estimate these models (Pinheiro et al. 2016). The long-term growth rate of a seedling (a factor with two levels: fast or slow) was included as an explanatory variable. Furthermore, two nested random factors were included: family and seedling identity (as three sample roots per seedling within a family were investigated as an individual observational unit).

Generalized additive mixed models (GAMMs) were used to investigate differences in root properties along zero order sample roots. The probability of occurrence of side branches, the number of root tips and all links, and the total length of links in branches along zero order links of sample roots were analyzed using the function *gamm* in library

mgcv in R (Wood 2004, 2006, 2011). Models for seedlings of fast- and slow-growing phenotypes were estimated separately. Models for the probability of occurrence of side branches were estimated assuming a quasi-binomial distribution with logit link function in R. The response in this model was a factor with two levels: 0 = a side branch does not exist but a root tip was visible, 1 = a side branch with more than one link existed. GAMMs for count responses (number of root tips, and all links per branch, i.e., both side branches and root tip branches were included) were estimated assuming a quasi-Poisson distribution with log link function, and the model for the total length of branches (mm) as a response was estimated with a log-linear model. As an explanatory variable we used the total length of sample root links of zero order in cm and in a separate model the total length was scaled from 0 to 100%. These variables were smoothed when the models were estimated. As random factors we used family, seedling identity (as three sample roots per seedling within a family were investigated as an individual observational unit), and identity of a sample root (as several side branches and root tips existed per sample root length). GAMMs are able to find curvilinear relationships between two variables, as in these models, tiny regression lines are combined together to form a smooth line representing variation in data along an investigated explanatory variable (Wood 2004, 2006, 2011). *P*-values for the curves are given, but no parametric estimate for the smoothed explanatory variable is provided as a result of this analysis. Statistically significant results can be visualized by drawing a response curve based on the GAMM.

Results

Seedling results

There were no differences in shoot height, specific root length, specific root area, root tissue density, the total biomass of shoots or roots, nor in root:shoot biomass ratio between

seedlings of fast- and slow-growing Norway spruce phenotypes (Table 3). Only total biomass of the three sample roots per seedling was indicatively higher in fast- than in slow-growing seedlings.

Sample root results

The thickest links (zero order) comprised less than 20% of the root links while more than 80% of all links were of the 1st to 5th order (Table 2). In our data, root diameter decreased with increasing order (note that we used the developmental order-based classification of counting orders outwards from the main branch of a sample root). Mean diameter and the length of links did not differ between the seedlings of fast- and slow-growing phenotypes although fast-growing seedlings showed slightly higher values. Half of the links were root tips while the rest were interior links.

Root volume of the sample roots was indicatively higher in fast- than in slow-growing seedlings (n = 162, p = 0.10), with predicted values based on the model being 319.7 ± 26.7 and 238.3 ± 26.7 mm^3, respectively. Also the number of side branches (single root tips excluded, GLMM, $p < 0.01$) and the total number of links were higher in fast- than in slow-growing seedlings (Tables 4-5, Fig. 2-4). In particular, first order interior links and root tips attached to them were more abundant in fast- than slow-growing seedlings (Table 5, Fig. 4F and K), yielding higher total length values for the first order links in fast-growing seedlings (LMM, p = 0.04). The surface area of root tips was higher in fast-growing seedlings (LMM, Table 4, Fig. 3L, p = 0.04), but root tip density (LMM, $p > 0.10$) and total length of the main root branch (of zero order) was similar in all seedlings (LMM, p = 0.20).

The angle of root tips, i.e., exterior links, was indicatively smaller in fast- (60.5°) than slow-growing seedlings (62.6°, LMM, p = 0.07), while the angle of other links,

i.e., interior links, was indicatively larger among fast- (31.4°) than slow-growing seedlings (30.2°, $p = 0.06$).

In the sample roots of fast-growing seedlings, the probability of occurrence of side branches was higher right next to the base and 10-15 cm further away along zero order root links compared to the branching profile of slow-growing seedlings (Figs. 5A and 6A). Moreover, the total number of root tips and links per branch, and the total length of all branch links along zero order roots peaked closer to the base in slow-growing seedlings (Fig. 5B-D), i.e., ca. 5 cm from the base, while in fast-growing seedlings these values remained relatively high up to 10-15 cm (Fig. 6B-D).

Discussion

The data supported our original hypothesis that seedlings of the fast-growing Norway spruce phenotypes have more branches further away along the sample roots than those with slow growth, although no differences in height or biomass between the groups could yet be observed. This indicates that fast-growing seedlings allocate more resources to root growth further away from the base of a seedling although – contrary to our hypothesis – the total length of zero root branches did not differ between the two growth phenotypes.

Longer branches and the larger number of side branches and root tips especially further away from the base of seedlings (see Figs. 2 and 5) suggest that fast-growing seedlings have higher potential to produce pioneer roots (Zadworny and Eissenstat 2011, Bagniewska-Zadworna et al. 2012), enabling the growth of higher number of absorbing fine roots. This may be a general characteristic that differentiates fast- from slow-growing phenotypes. The formation of branches in roots is a vital process as it enables plants to extend to a larger area and to increase their contact area with the soil (Pagès 2014, Beidler et al. 2015). When comparing various deciduous and evergreen tree species, Comas and Eissenstat

(2004) found that roots of fast-growing tree species had a greater degree of branching, but smaller diameters than slow-growing species. In our study, specific root length, SRL, did not differ between the seedlings of fast- and slow-growing phenotypes, although higher SRL values usually indicate thinner roots and greater root surface area (via longer roots with more branches) for nutrient absorption, while lower values indicate thicker roots with lower surface area (Craine et al. 2001, Comas and Eissenstat 2004, Maherali 2014, Weemstra et al. 2016). Interestingly, Ostonen et al. (2013) also did not find variation in SRL in Norway spruce along a latitudinal gradient. Most likely SRL is not an essential character regulating performance in spruce under different conditions. In Scots pine (*Pinus sylvestris* L.), however, SRL is dependent on latitude and, more specifically, on mean annual temperature (Ostonen et al. 2017, Zadworny et al. 2017).

In fast-growing seedlings, a larger branching angle of lower level interior links may relate to the ability to enlarge the soil contact area, while smaller angles of root tips may indicate a strategy of roots for more explorative growth further away from the base of a seedling in forest soils to better reach patches of nutrients (Beidler et al. 2015) and soil microbes (Pennanen et al. 1999) that may enable fast-growing seedlings to gather more resources for future growth. Especially, in combination with the observation that fast-growing spruce seedlings bore more side branches further away from the base of the seedling, their ability to gain water, nutrients and symbiotic associations in highly heterogeneous forest soils may be significantly higher than that of slow-growing ones since, e.g., ectomycorrhizal fungi often occur in patches 3 - 17 m apart (Lilleskov et al. 2004).

Ectomycorrhizal trees are more dependent on mycorrhizal hyphal foraging (i.e., in absorbing water and acquiring nutrients) than arbuscular mycorrhizal trees which invest more carbon in constructing foraging roots (Eissenstat et al. 2015, Chen et al. 2016). Furthermore, fertilization may increase root proliferation and reduce mycorrhizal

colonization (Eissenstat et al. 2015). Clear differences in fine root architecture between fast- and slow-growing Norway spruce origins have, thus far, only been found under mycorrhiza-free conditions in a study by Boukcim and Plassard (2003). They reported that the number and length of root tips were higher in a fast-growing spruce family, but the difference disappeared when roots were associated with an ectomycorrhizal fungus, i.e., it seemed that after mycorrhizal colonization, the fast-growing family did not anymore allocate resources to root growth. Our results were obtained under conventional growth conditions of spruce, and seedlings were cultivated in a substrate containing soil humus ensuring ectomycorrhizal colonization of the roots (> 81%, ca. five species per seedling with no differences between the seedlings of fast- and slow-growing phenotypes; Velmala et al. 2014b). Interestingly, in our study, fast-growing seedlings invested differently to roots than slow-growing seedlings, i.e., they had higher number of side branches, links and root tips, even after ectomycorrhizal colonization. As seedlings were cultivated under uniform conditions, the observed differences in root architecture most probably reflect variation in the genetic background of the spruce seedlings rather than environmental effects (see Fitter and Stickland 1991).

In contrast to studies by Velmala et al. (2014a, b) and our hypothesis, we showed that root tip density does not differ between seedlings of fast- and slow-growing spruce phenotypes, although the number of root tips was higher in fast-growing seedlings. This may be due to the finding that also the total length of links was indicatively higher in fast-growing seedlings, thus resulting in similar densities between the phenotypes. Velmala et al. (2014a) reported that the root tip density of younger, 13-month-old Norway spruce seedlings grown in a homogenous growth substrate in a nursery was higher and that the roots were denser in seedlings of slow-growing phenotype, but also that the total number of root tips in a seedling did not differ. However, in Norway spruce at 12 years of age, roots of fast-growing clones supported higher ectomycorrhizal diversity and a greater number of root tips

than the slow-growing ones (Korkama et al. 2006, 2007). Differences in these results estimating root tip densities may reflect either true differences in roots at different stages of seedling growth or different methods used to measure root tip densities. The WinRHIZO™ analysis used here provided an estimate of large compartments of root systems compared to previous studies in which roots were sampled by a soil corer (Korkama et al. 2006) or cut into 1-2 cm pieces (Velmala et al. 2013) as sampling was aimed only to detect ectomycorrhizal root tips. Still, both the mycorrhizal root tip-focused sampling and the present root architecture approach show that tree genotype affects root structure. This indicates that different strategies for root investigations are possible if limitations of the approach and comparability of the samples between treatments are considered.

This is the first time the spatial distribution of root branch properties, i.e., side roots and root tips along main side branches, was investigated. We believe that this is an approach that could be utilized in future studies when complete root systems of small seedlings can be analysed. Furthermore, the use of developmental order-based classification allowed us to get detailed information on the location of root variables, such as side branches and root tips, within the root systems, e.g., along the main root branch of zero order. However, due to the limited number of observations, our results were often indicative, and therefore larger datasets are needed to provide a comprehensive view of the belowground development of tree seedlings.

Conclusions

The roots of 16-month-old seedlings of Norway spruce phenotypes with contrasting growth rates in long-term field experiments were investigated. After two growing seasons, aboveground parts of seedlings were still even-sized, but the differing growth phenotypes had different strategies for early root growth under uniform conditions. Fast-growing phenotypes

showed higher numbers of root tips (i.e., exterior links) and interior links, and oriented and allocated root tips and biomass further away from the base of the seedling than slow-growing ones. The genetic background of spruce seedlings is likely to determine these differences since environmental conditions were similar for all seedlings. The ability of different spruce origins to control their root architecture may cause, in later life, differences in their ability to gain nutrients, water and mutualistic associations in heterogeneous forest soils, potentially explaining the systematic long-term growth differences and competitive relationships in forest ecosystems. We conclude that over time, small differences in these specific allocation patterns for root growth may create positive self-reinforcing mechanisms that underlie the higher growth rates and superior long-term growth performance of some trees in forests.

Conflict of Interest

None declared.

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Figure legends

Figure 1. Sampling design: (A) Altogether 54 seedlings from six spruce families (i.e., nine seedlings per family), and their sample roots, three per seedling, were investigated. (B) Schematic representation of a sample root with explanations for some root variables. A link is a part of a root between two branches or the most distant part of a branch (i.e., root tip). All side branches include more than one root link whereas a root tip includes one link only. Angle is determined as degrees between two links starting from the same point. The order of branches (and links within a branch) is indicated with a number as presented in the figure. The zero order root links form the main root branch of a sample root, first order links form a first order branch originating from the zero order root branch, second order links form a second order branch originating from first order branch links etc. This developmental order-based classification was utilized in this study.

Figure 2. Schematic model of four sample root branches of Norway spruce (*Picea abies*) seedlings representing: (A) a sample root with the highest number of links for fast-growing seedlings, (B) a root containing a median number of root links for fast-growing seedlings, (C) a root containing a median number of root links for slow-growing seedlings, and (D) a sample root with the lowest number of links for slow-growing seedlings. The lengths, widths and branching angles of the root links were analyzed with WinRHIZO™, and visual rendering was done using the LIGNUM program (Sievänen et al. 2010).

Figure 3. Number, total length and surface area of links among the sample roots of fast- and slow-growing Norway spruce (*Picea abies*) seedlings based the predictions of linear or generalized linear mixed models (n = 162). Values for the whole sample root, side branches,

interior links, i.e., other links than root tips, and root tips, i.e., exterior links are presented. Standard error values are not presented as these are not available for all model types. Statistically significant differences ($p < 0.05$) are indicated with an asterisk, and mean raw data values for spruce families are shown with a cross. See Table 4.

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602 in cm for fast- and slow-growing Norway spruce seedlings. All responses are statistically
603 significantly curvilinear (GAMMs, $p < 0.01$) and 95% confidence intervals are presented as
604 dash line response curves.

Tables

Table 1. Origin, seed collection year and growth performance of the Norway spruce (*Picea abies* (L.) Karst.) families used in this study. The growth performance of two groups (fast and slow) has been assessed from 14 year old trees in seven to 10 growth experiments done earlier in the field. The table is modified from Velmala et al. (2014a).

Family code	Origin [a]		Collection year	Growth performance [b]	Succession rate [c]	Group
	Female	Male				
298	239	1515	2000	Good	89	Fast
612	5519	4309	1998	Excellent	102	Fast
1162	46	Open pollination	1989	Excellent	95	Fast
394	7133	Open pollination	1983	Poor	33	Slow
427	5514	Open pollination	1983	Poor	35	Slow
1183	217	Open pollination	1989	Poor	33	Slow

[a] All trees are of southern Finnish origin, except male 4309 which originates from Germany.
[b] Growth performance of the family was based on stem height (see Venäläinen 1993).
[c] Proportional reference level is 50: values more or less than 50 indicate better or worse aboveground growth than average in Norway spruce, respectively.

Table 2. Mean diameter and length of root links, and the proportion of root tips and other links in fast- and slow-growing Norway spruce seedlings (*Picea abies*) based on raw data gathered from WinRHIZO (n = 162). Means with standard deviations (SD) are presented.

Variable	Fast (n = 81)	Slow (n = 81)
Mean diameter of links (mm)		
• All links	0.51 ± 0.08	0.50 ± 0.06
• Interior links [a]	0.65 ± 0.11	0.64 ± 0.08
• Root tips [a]	0.37 ± 0.05	0.35 ± 0.04
• 0 order links [b]	0.95 ± 0.21	0.90 ± 0.16
• 1st order links	0.49 ± 0.08	0.47 ± 0.08
• 2nd order links	0.37 ± 0.06	0.36 ± 0.06
• 3rd to 5th order links	0.35 ± 0.11	0.33 ± 0.12
Mean length of links (mm)		
• All links	2.95 ± 0.44	2.91 ± 0.52
• Interior links	2.93 ± 0.53	2.91 ± 0.64
• Root tips	2.98 ± 0.49	2.91 ± 0.54
• 0 order links	3.26 ± 0.73	3.32 ± 0.87
• 1st order links	3.68 ± 0.64	3.52 ± 0.73
• 2nd order links	2.15 ± 0.54	2.09 ± 0.46
• 3rd to 5th order links	1.02 ± 0.41	1.05 ± 0.43
Proportion of links (%) [c]		
• Interior links	51.40 ± 0.01	51.39 ± 0.01
• Root tips	48.60 ± 0.01	48.61 ± 0.01
• 0 order links	16.52 ± 5.80	17.77 ± 5.85
• 1st order links	43.90 ± 3.84	44.48 ± 4.02
• 2nd order links	33.04 ± 5.75	31.93 ± 5.59
• 3rd to 5th order links	6.55 ± 4.25	5.82 ± 4.42

[a] Fitter and Stickland (1991) used the terms exterior links for root tips and interior links for other links than root tips

[b] Note that the developmental order-based classification was used. Zero order links form the main branch of a sample root, first order links form branches originating from zero order links, second order links form branches originating from first order links etc. (see Fig. 1b). 3rd to 5th order links occurred in 100% and 94% of the roots of fast- and slow-growing seedlings, respectively

[c] The proportion of interior or root tip links or a particular root order to the total number of links

Table 3. Differences in some variables describing growth between the fast- and slow-growing Norway spruce seedlings (*Picea abies*). Predicted values are based on linear mixed models (LMM) (n = 54). Means with standard errors (SE) are presented.

Variable	Fast (n = 27)	Slow (n = 27)	p
Shoot height (mm)	156.7 ± 12.6	145.6 ± 10.5	0.54
Specific root length (SRL, m g^{-1})	68.5 ± 3.5	70.8 ± 3.5	0.66
Specific root area, (SRA, m^2 kg^{-1})	112.5 ± 5.7	111.4 ± 5.7	0.90
Root tissue density, (RTD, kg m^{-3})	61.0 ± 4.9	57.6 ± 4.9	0.66
Total biomass of shoots (g) [a]	0.903 ± 0.105	0.817 ± 0.105	0.59
Total biomass of roots (g) [a]	0.245 ± 0.025	0.229 ± 0.025	0.68
Root:shoot biomass ratio [a]	0.29 ± 0.02	0.28 ± 0.02	0.78
Total biomass of the three sample roots per seedling (g)	0.052 ± 0.004	0.040 ± 0.004	0.09

[a] Number of seedlings both in the fast- and slow-growing group was nine.

Table 4. Linear or generalized linear mixed models (LMMs and GLMMs, respectively) results relating to root characteristics of the sample roots of fast- and slow-growing Norway spruce (*Picea abies*) seedlings (n = 162). LMMs were used for the analysis of total length and the surface area of links, and GLMMs for the number of links. Statistically significant differences ($p < 0.05$) between fast- and slow-growing seedlings are in bold. Coefficients with standard errors (SE) are shown. See Fig. 3.

Variable	Intercept		Difference between the phenotypes (slow vs. fast)	
	Coeff. ± SE	*p*	Coeff. ± SE	*p*
Number of links				
• All links	5.652 ± 0.075	< 0.01	0.251 ± 0.106	**0.02**
• All branches [a]	5.469 ± 0.082	< 0.01	0.273 ± 0.116	**0.02**
• Interior links [b]	4.988 ± 0.077	< 0.01	0.251 ± 0.108	**0.02**
• Root tips [b]	4.931 ± 0.074	< 0.01	0.251 ± 0.105	**0.02**
Total length of links (mm)				
• All links	865.620 ± 76.147	< 0.01	255.363 ± 107.688	0.08
• All branches [a]	713.075 ± 72.847	< 0.01	239.498 ± 103.021	0.08
• Interior links [b]	444.905 ± 42.182	< 0.01	126.845 ± 59.655	0.10
• Root tips [b]	420.715 ± 36.266	< 0.01	128.518 ± 51.287	0.07
Surface area of links (mm^2)				
• All links	1409.306 ± 126.738	< 0.01	444.250 ± 179.235	0.07
• All branches [a]	990.104 ± 101.524	< 0.01	369.317 ± 143.577	0.06
• Interior links [b]	883.485 ± 83.222	< 0.01	243.814 ± 117.693	0.11
• Root tips [b]	525.821 ± 47.255	< 0.01	200.436 ± 66.829	**0.04**

[a] Side branches and root tips (see Fig. 1b).
[b] Fitter and Stickland (1991) used the terms exterior links for root tips and interior links for other links than root tips.

Table 5. Generalized linear mixed model (GLMMs) results relating to the number of links of the sample roots of fast- and slow-growing Norway spruce (*Picea abies*) seedlings in different root orders (n = 162). Statistically significant differences ($p < 0.05$) between fast- and slow-growing seedlings are in bold. Coefficients with standard errors (SE) are shown. See Fig. 4.

Variable	Intercept		Difference between the phenotypes (slow vs. fast)	
	Coeff. ± SE	*p*	Coeff. ± SE	*p*
Total number of links [a]				
• 0 order links	3.788 ± 0.052	< 0.01	0.128 ± 0.073	0.08
• 1st order links	4.827 ± 0.068	< 0.01	0.231 ± 0.096	**0.02**
• 2nd order links	4.538 ± 0.091	< 0.01	0.301 ± 0.128	**0.02**
• 3rd to 5th order links	2.790 ± 0.191	< 0.01	0.394 ± 0.269	0.14
Number of interior links [b]				
• 0 order links	3.764 ± 0.053	< 0.01	0.132 ± 0.075	0.08
• 1st order links	4.383 ± 0.084	< 0.01	0.265 ± 0.119	**0.03**
• 2nd order links	2.793 ± 0.162	< 0.01	0.336 ± 0.228	0.14
• 3rd to 4th order links	0.443 ± 0.250	0.08	0.316 ± 0.345	0.36
Number of root tips [b]				
• 0 order links	1		1	-
• 1st order links	3.778 ± 0.054	< 0.01	0.131 ± 0.076	0.08
• 2nd order links	4.328 ± 0.082	< 0.01	0.285 ± 0.115	**0.01**
• 3rd to 5th order links	2.691 ± 0.186	< 0.01	0.397 ± 0.263	0.13

[a] Note that the developmental order-based classification was used. Zero order links form the main branch of a sample root, first order links form branches originating from zero order links, second order links form branches originating from first order links etc. (see Fig. 1b).

[b] Fitter and Stickland (1991) used the terms exterior links for root tips and interior links for other links than root tips.

Figures

Figure 1

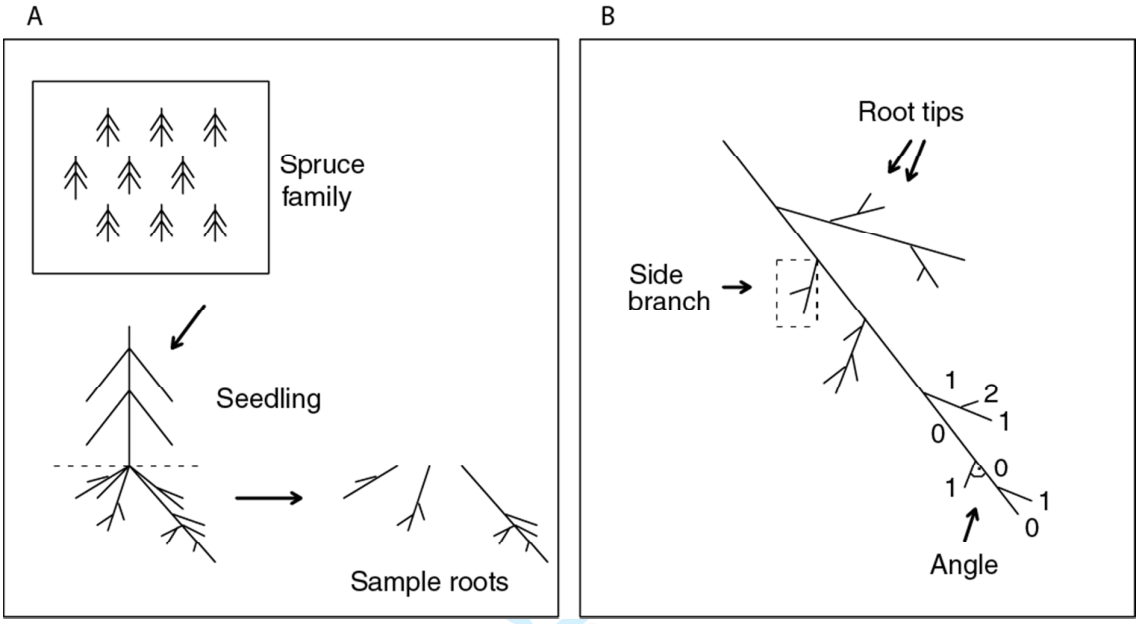

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Figure 2

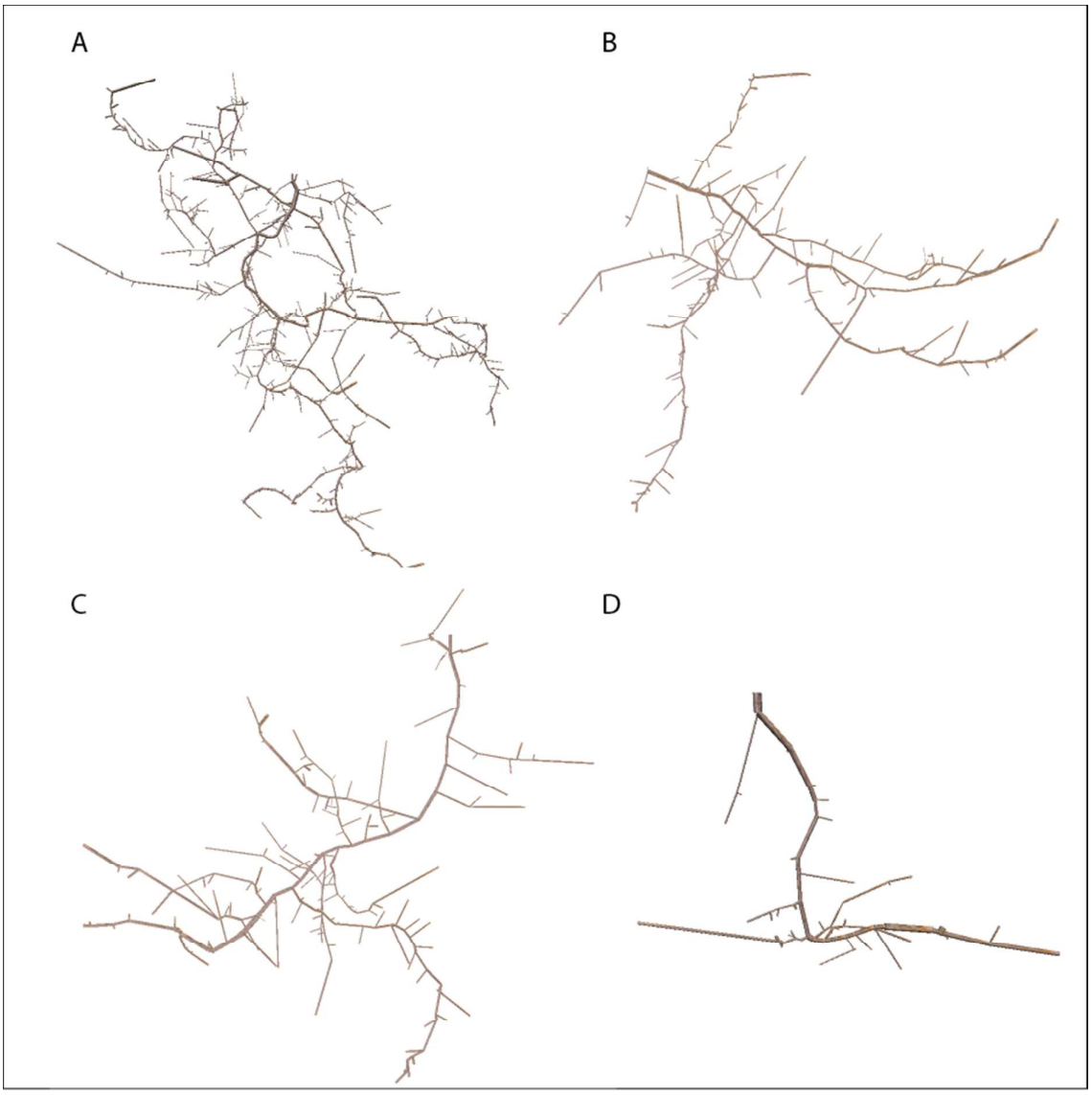

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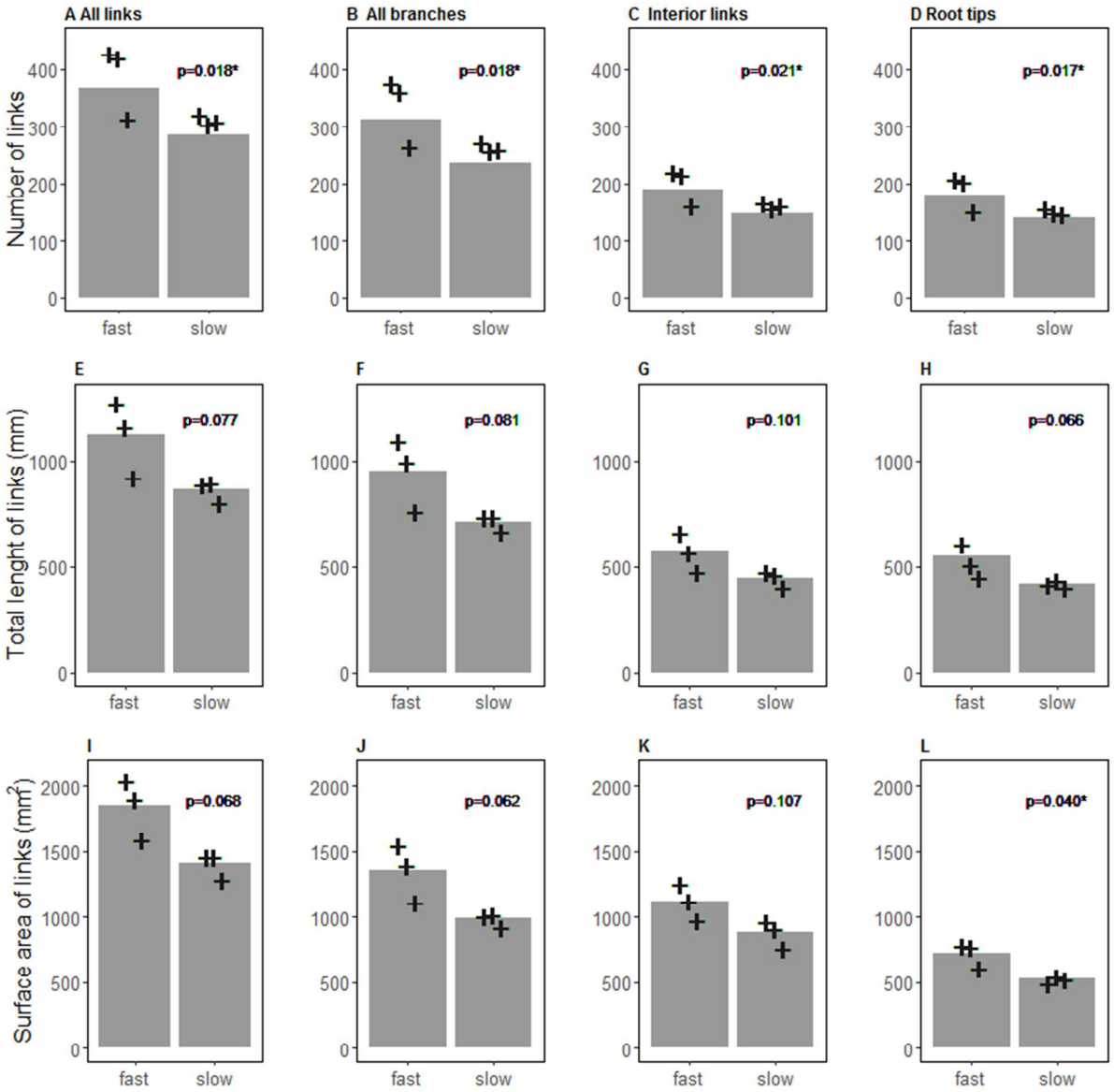
Figure 3


A All links
B All branches
C Interior links
D Root tips
E
F
G
H
I
J
K
L
p=0.018*
p=0.018*
p=0.021*
p=0.017*
p=0.077
p=0.081
p=0.101
p=0.066
p=0.068
p=0.062
p=0.107
p=0.040*
Number of links
Total lenght of links (mm)
Surface area of links (mm²)
fast
slow
0
100
200
300
400
500
1000
1500
2000

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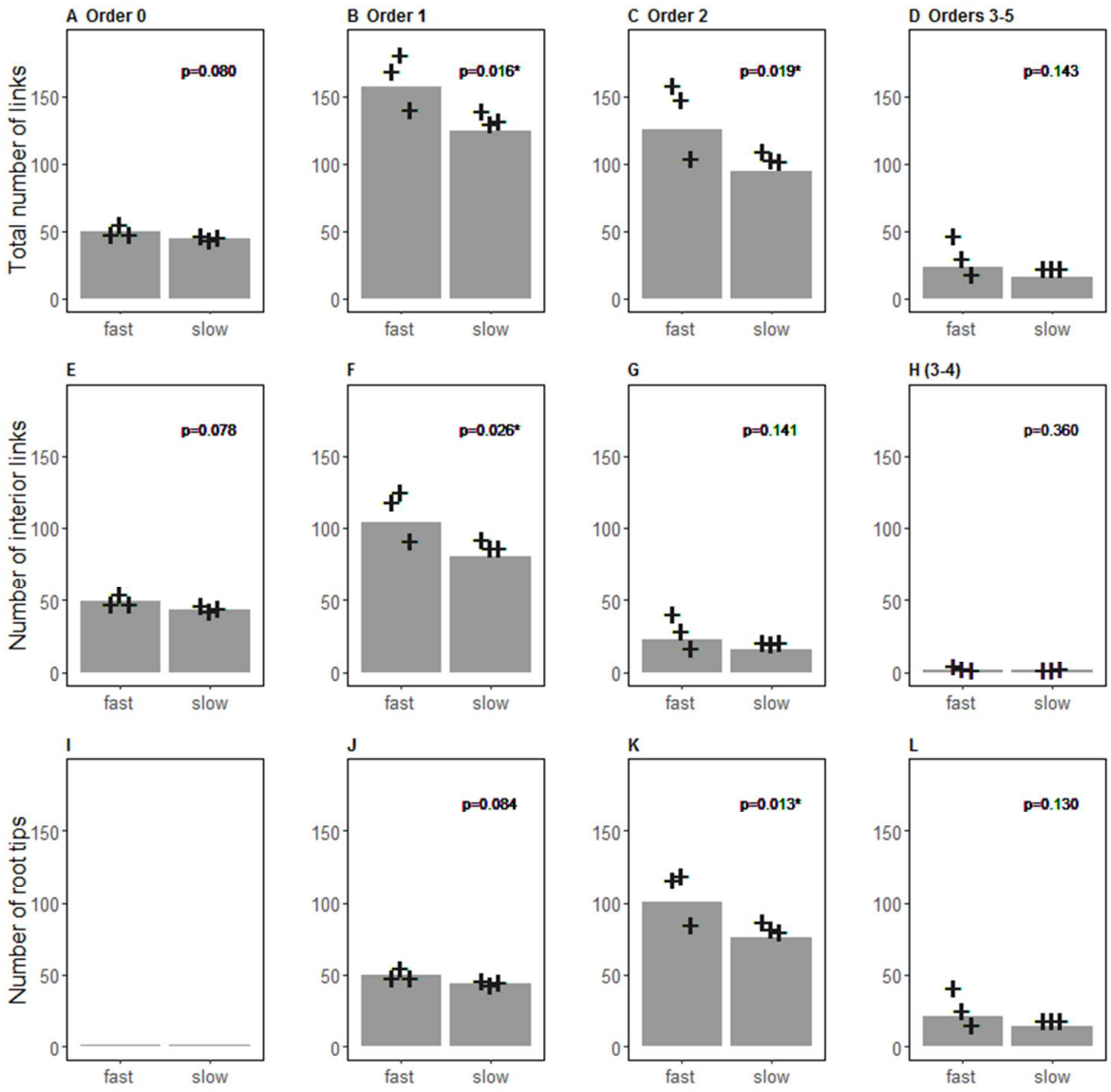
A Order 0
B Order 1
C Order 2
D Orders 3-5
p=0.080
p=0.016*
p=0.019*
p=0.143
Total number of links
E
F
G
H (3-4)
p=0.078
p=0.026*
p=0.141
p=0.360
Number of interior links
I
J
K
L
p=0.084
p=0.013*
p=0.130
Number of root tips
0
50
100
150
fast
slow

Figure 4. Total number of links, the number of interior links (i.e., other than root tips), and root tips between the sample roots of fast- and slow-growing seedlings of Norway spruce (*Picea abies*) based on the predicted values of generalized linear mixed models (GLMMs, $n = 162$). Values for different root orders are presented (note that the developmental order-based classification was used, see Fig. 1). Note that the numbers are extremely low in panels H and I. Standard error values are not presented as these are not available for this model type. Statistically significant differences ($p < 0.05$) between the fast- and slow-growing seedlings are indicated with an asterisk, and mean raw data values for spruce families are shown with a cross. See Table 5.

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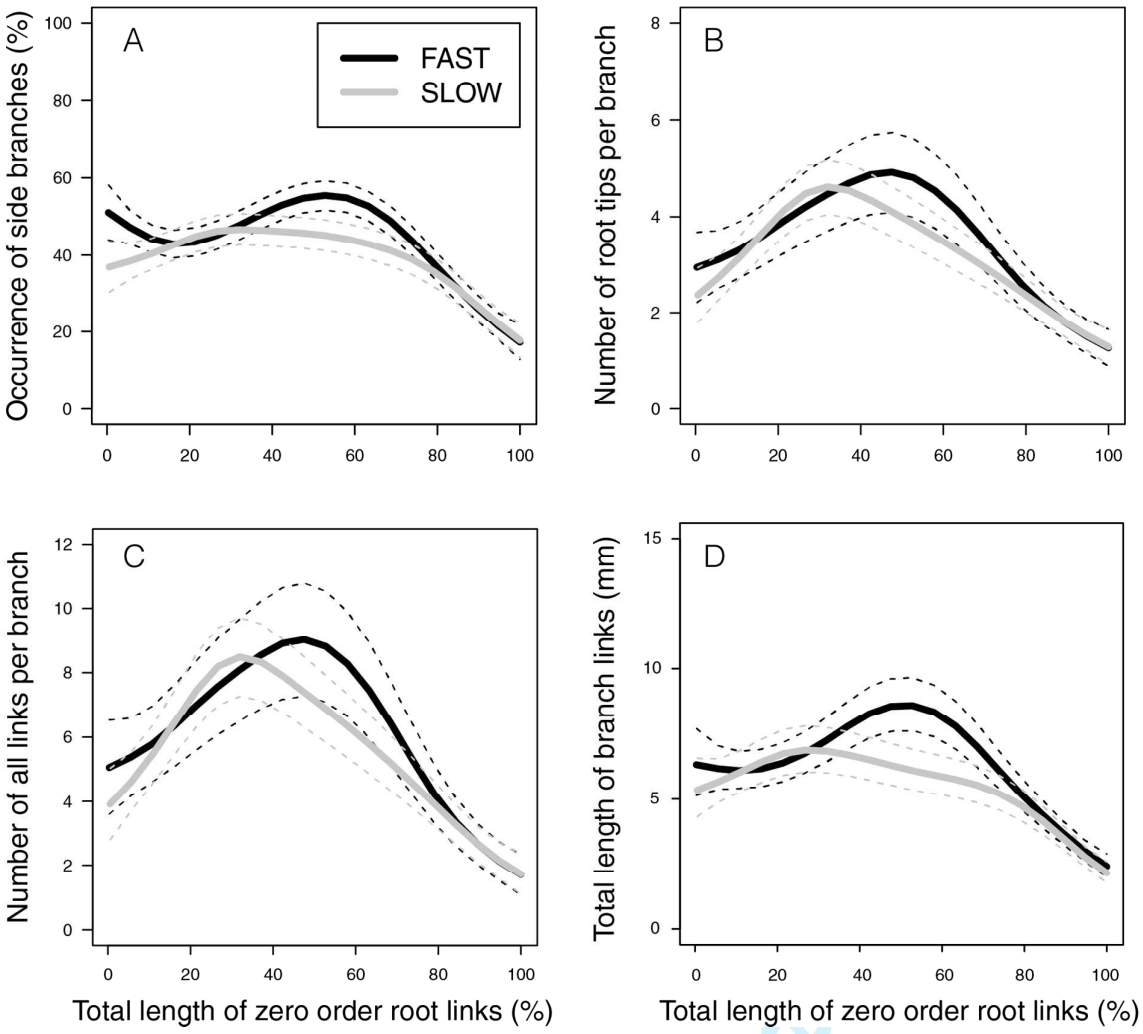

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Figure 6

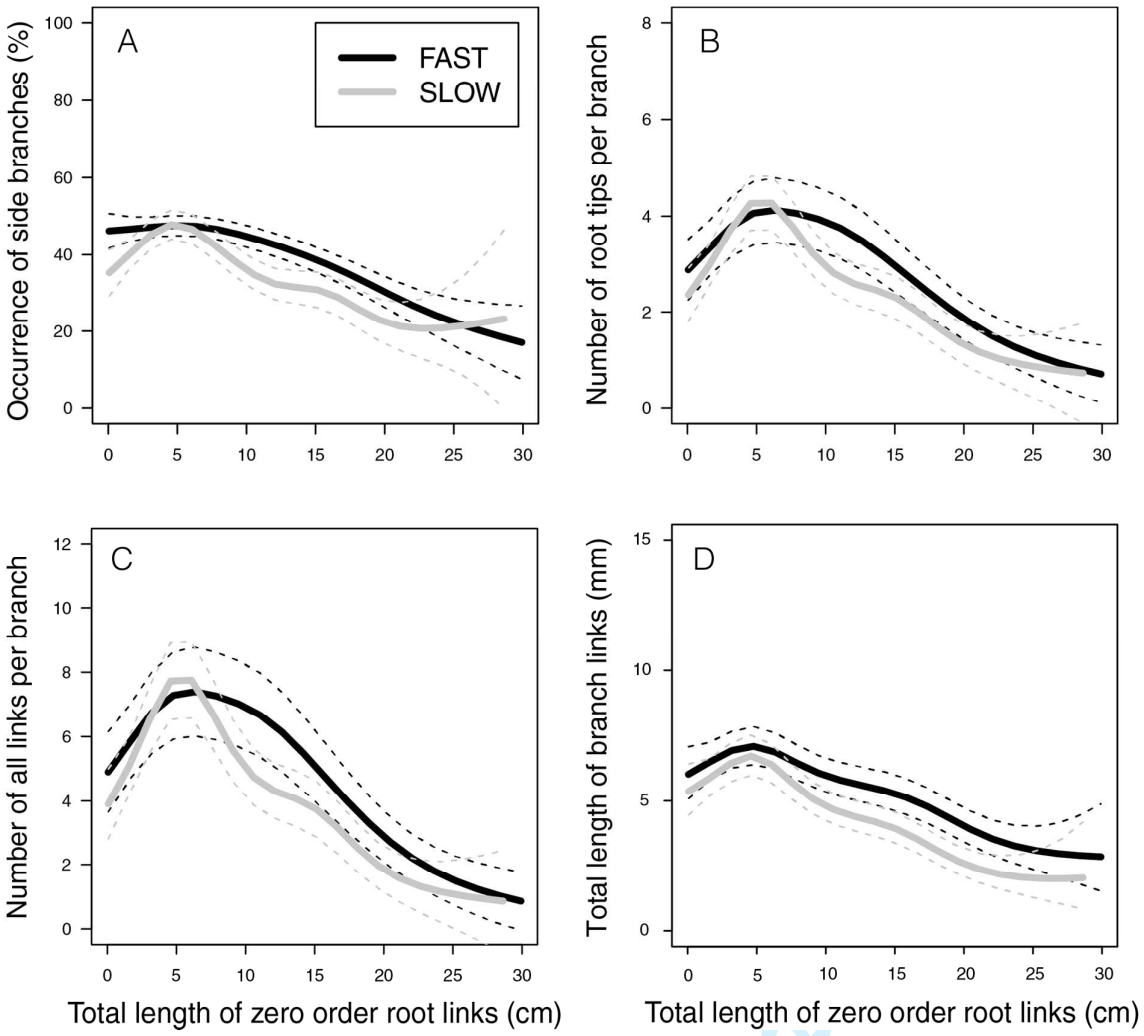

Figure 6 (A) The probability of occurrence of side branches, (B) the number of root tips per branch, (C) the number of all links per branch (composed of one or more links), and (D) the total length of branch links along the total length of sample root links of zero order expressed in cm for fast- and slow-growing Norway spruce seedlings. All responses are statistically significantly curvilinear (GAMMs, $p < 0.01$) and 95% confidence intervals are presented as dash line response curves.

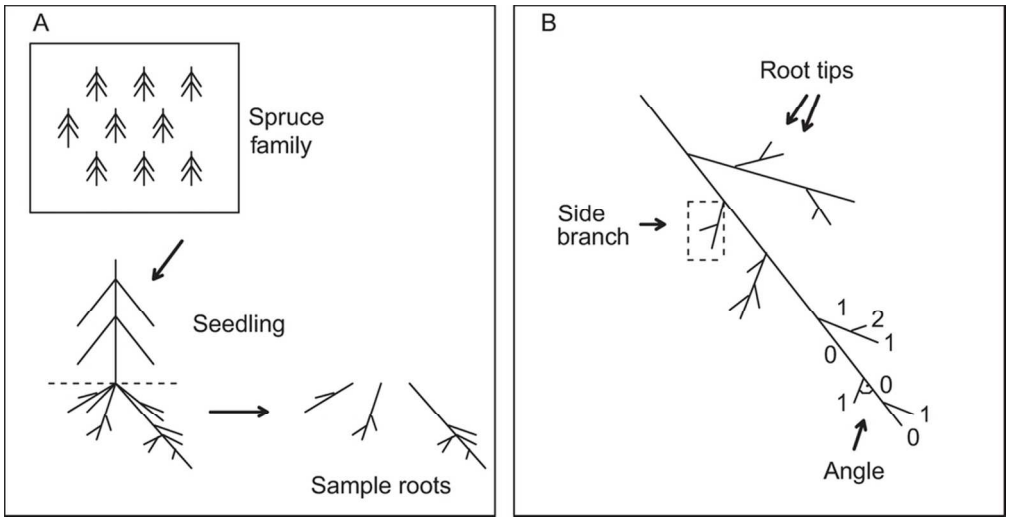

Figure 1. Sampling design: (A) Altogether 54 seedlings from six spruce families (i.e., nine seedlings per family), and their sample roots, three per seedling, were investigated. (B) Schematic representation of a sample root with explanations for some root variables. A link is a part of a root between two branches or the most distant part of a branch (i.e., root tip). All side branches include more than one root link whereas a root tip includes one link only. Angle is determined as degrees between two links starting from the same point. The order of branches (and links within a branch) is indicated with a number as presented in the figure. The zero order root links form the main root branch of a sample root, first order links form a first order branch originating from the zero order root branch, second order links form a second order branch originating from first order branch links etc. This developmental order-based classification was utilized in this study.

88x45mm (300 x 300 DPI)

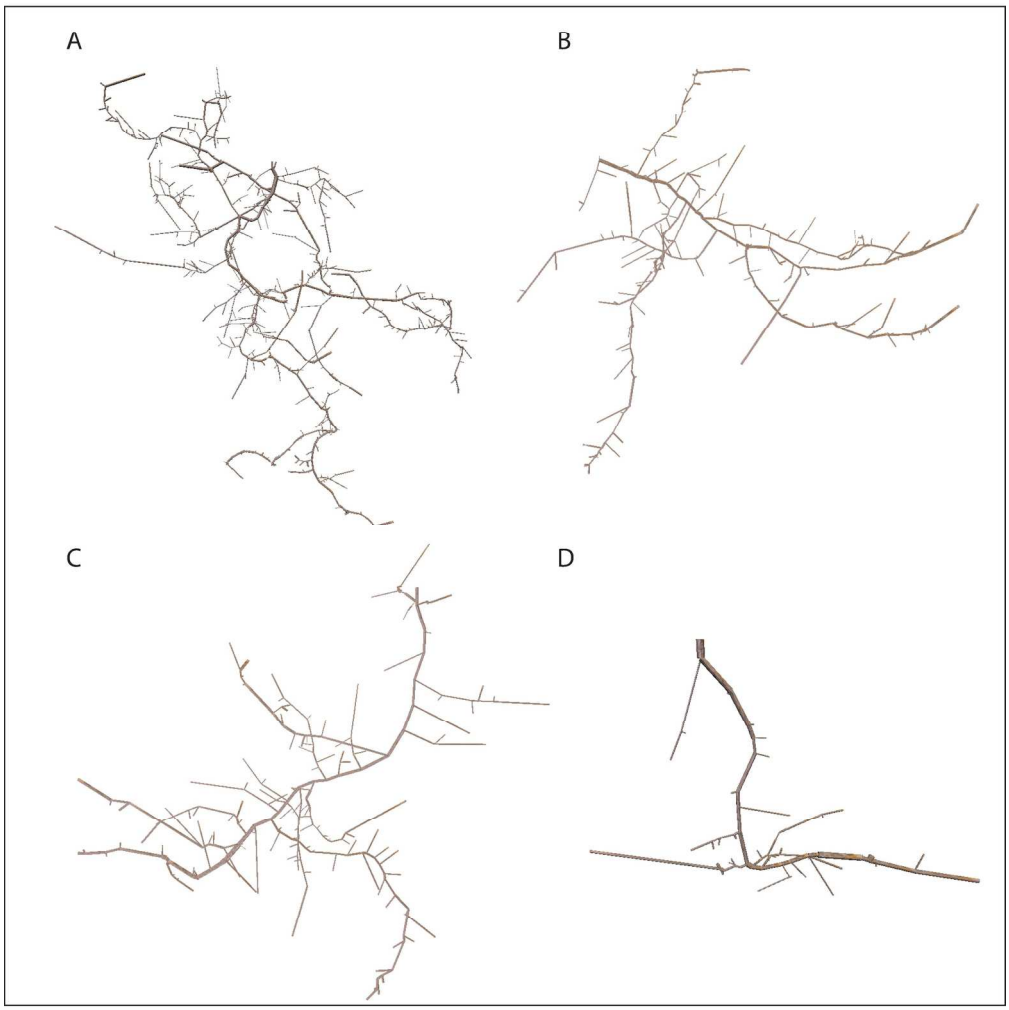

Figure 2. Schematic model of four sample root branches of Norway spruce (Picea abies) seedlings representing: (A) a sample root with the highest number of links for fast-growing seedlings, (B) a root containing a median number of root links for fast-growing seedlings, (C) a root containing a median number of root links for slow-growing seedlings, and (D) a sample root with the lowest number of links for slow-growing seedlings. The lengths, widths and branching angles of the root links were analyzed with WinRHIZO™, and visual rendering was done using the LIGNUM program (Sievänen et al. 2010).

172x186mm (300 x 300 DPI)

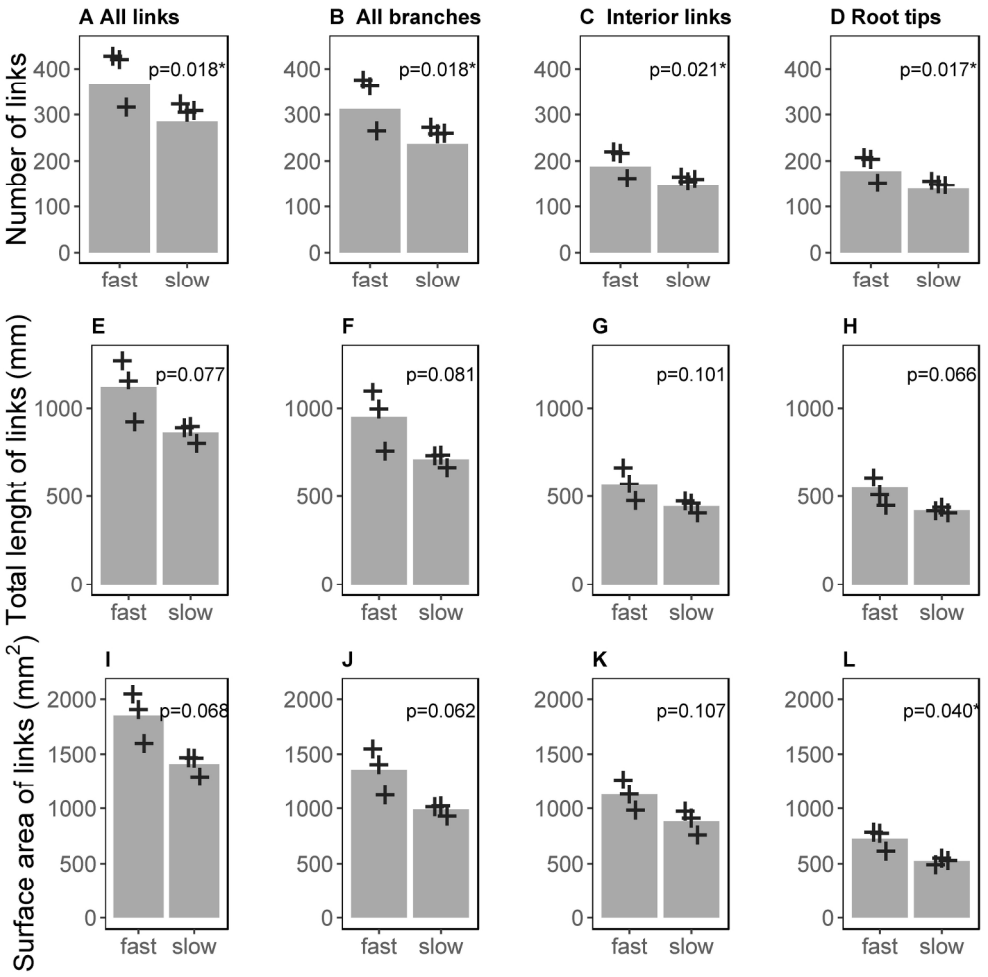

Figure 3. Number, total length and surface area of links among the sample roots of fast- and slow-growing Norway spruce (Picea abies) seedlings based the predictions of linear or generalized linear mixed models (n = 162). Values for the whole sample root, side branches, interior links, i.e., other links than root tips, and root tips, i.e., exterior links are presented. Standard error values are not presented as these are not available for all model types. Statistically significant differences ($p < 0.05$) are indicated with an asterisk, and mean raw data values for spruce families are shown with a cross. See Table 4.

203x203mm (300 x 300 DPI)

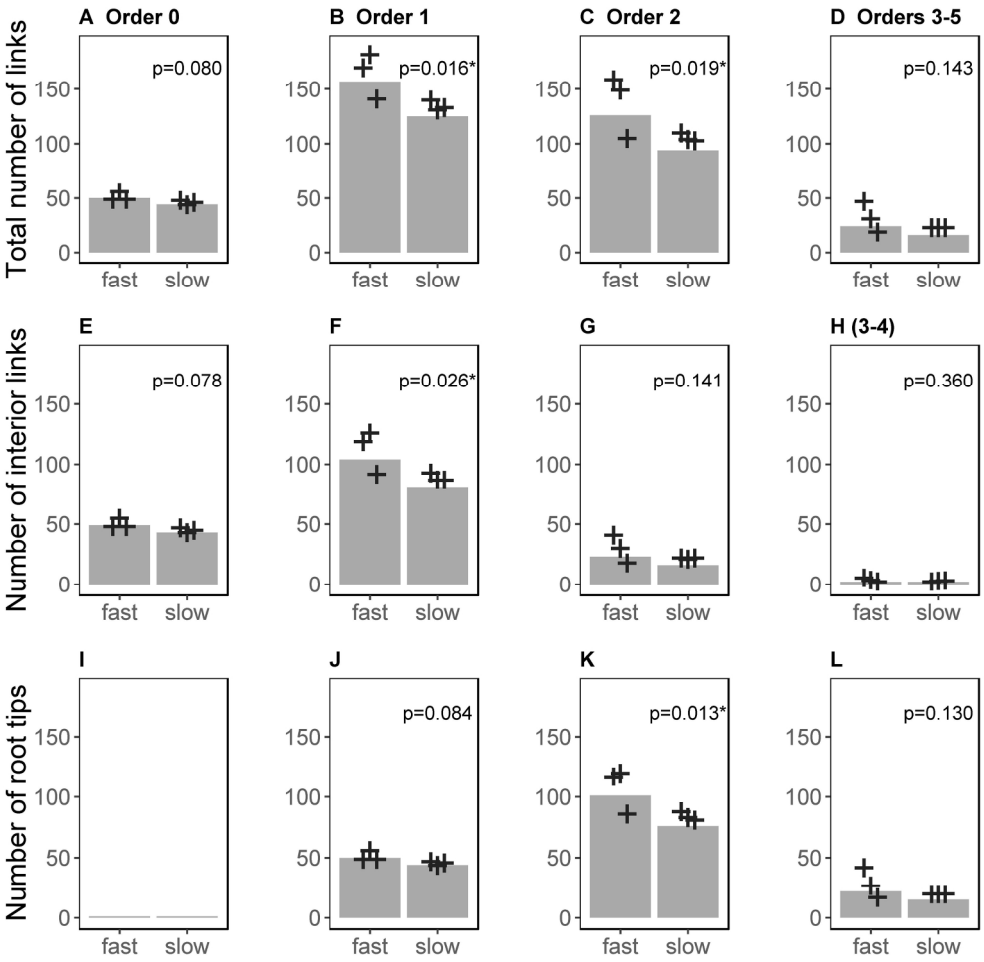

Figure 4. Total number of links, the number of interior links (i.e., other than root tips), and root tips between the sample roots of fast- and slow-growing seedlings of Norway spruce (Picea abies) based on the predicted values of generalized linear mixed models (GLMMs, n = 162). Values for different root orders are presented (note that the developmental order-based classification was used, see Fig. 1). Note that the numbers are extremely low in panels H and I. Standard error values are not presented as these are not available for this model type. Statistically significant differences ($p < 0.05$) between the fast- and slow-growing seedlings are indicated with an asterisk, and mean raw data values for spruce families are shown with a cross. See Table 5.

203x203mm (300 x 300 DPI)

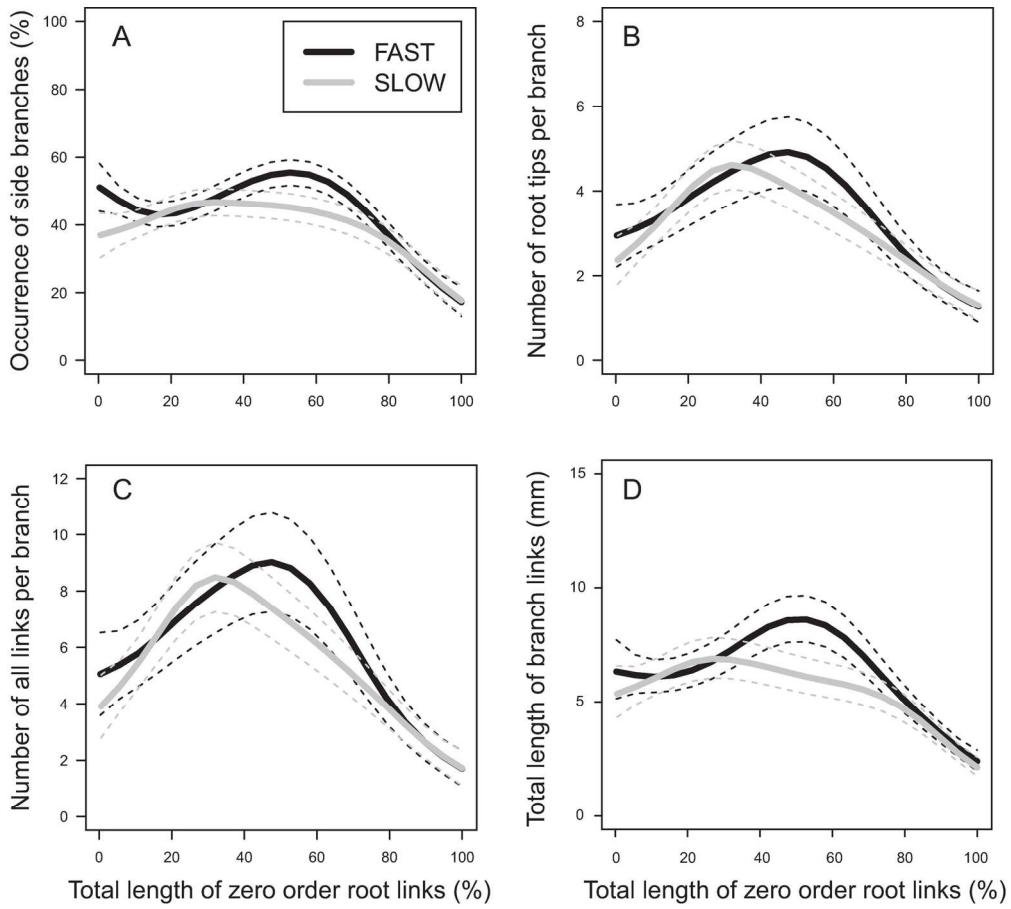

Figure 5 (A) The probability of occurrence of side branches, (B) the number of root tips per branch (composed of one or more links), (C) the number of all links per branch, and (D) the total length of branch links along the total length of sample root links of zero order expressed as a proportion (0-100%) for fast- and slow-growing Norway spruce seedlings. All responses are statistically significantly curvilinear (GAMMs, $p < 0.01$) and 95% confidence intervals are presented with dashed lines.

157x141mm (300 x 300 DPI)

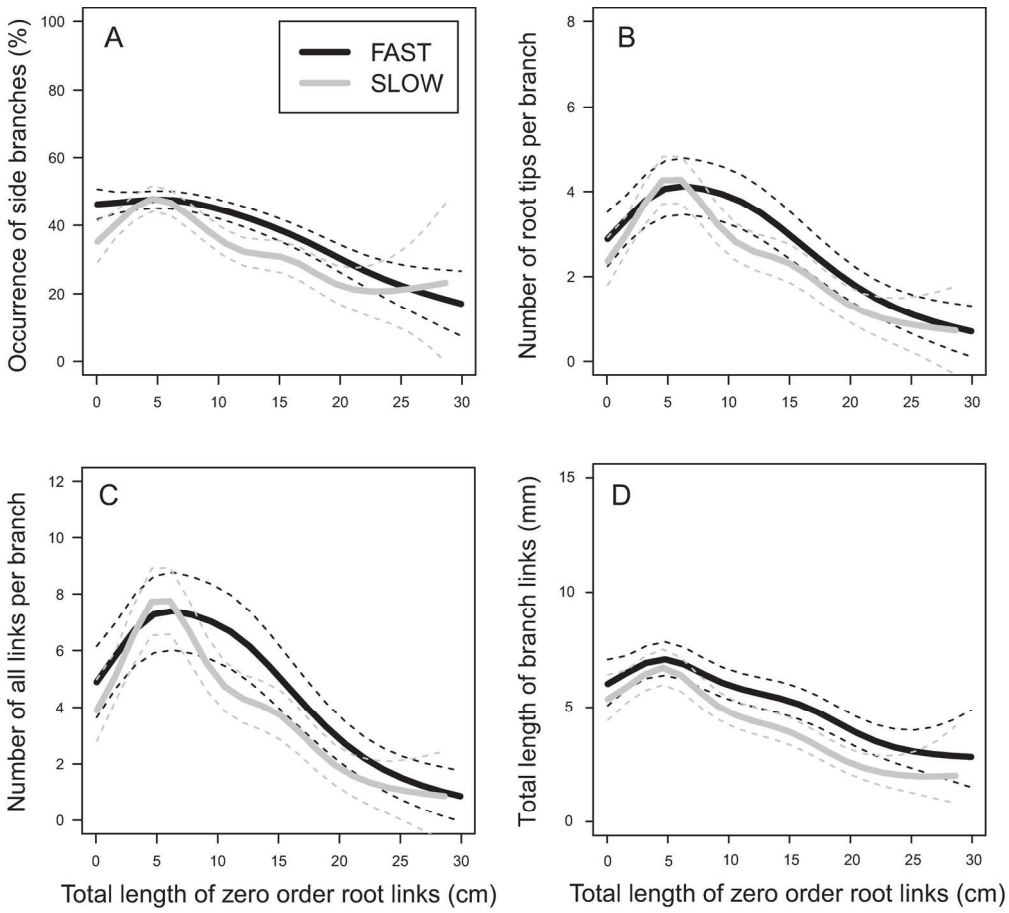

Figure 6 (A) The probability of occurrence of side branches, (B) the number of root tips per branch, (C) the number of all links per branch (composed of one or more links), and (D) the total length of branch links along the total length of sample root links of zero order expressed in cm for fast- and slow-growing Norway spruce seedlings. All responses are statistically significantly curvilinear (GAMMs, $p < 0.01$) and 95% confidence intervals are presented as dash line response curves.

157x142mm (300 x 300 DPI)